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Screening of Alpha Amylase Inhibitory Activity and Antioxidant Activity of Selected Sri Lankan Medicinal Plants and Development of Herbal Tea

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Authors' contributions

This work was carried out in collaboration among all authors. Author AGAWA designed the study, performed the statistical analysis, wrote the protocol, supervised laboratory work and wrote the first draft of the manuscript. Author JFU performed laboratory work and preliminary data analysis under the supervision of author AGAWA. Author KGBAS supported in literature survey, data analysis and manuscript writing. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Herbal medicines are widely used because of its health benefits and minimum side effects. There are many underutilized medicinal herbs in Sri Lanka which are not subjected to any scientific study to prove their health benefits. The present study attempted to identify the health benefits and possibility to develop herbal teas from selected herbs; *Solanum trilobatum, Ocimum tenuiflorum, Cardiospermum halicacabum, Acalypha indica* and *Plectranthus amboinicus.*
Study Design: Complete Randomized Design was employed.
Place and Duration of Study: The study was carried out in Agricultural Chemistry Laboratory of Uva Wellassa University, Badulla, Sri Lanka From November 2018 to March 2019.
Methodology: Leaf samples of these herbs were oven dried for 12 hours at 50℃. Infusions were prepared by boiling varying amounts of leaf samples from 1g to 5g in 100 ml of distilled water and tested for antioxidant activity with DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay and α-amylase inhibition activity using Dinitrosalicylic acid method. Sensory evaluation was conducted for the same

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infusions with ISO-3103 specifications to select the best amounts of samples for consumption. The selected amounts were subjected to chemical analysis.
Results: The best antioxidant activity and α-amylase inhibition activity were observed in *C.halicacabum* with 7.71 mg ml^{-1} and 45.51 mg ml^{-1} IC_{50} values respectively. Results confirmed that all leaf infusions contain Anthocyanin (4.20-0.11mg dm^{-3}), Polyphenols (120.49-32.93 mg dm^{-3}) and Free Sugar (262.95-24.67mg dm^{-3}).
Conclusion: Selected herbs show positive responses towards selected biochemical properties and there is a very good potential to develop herbal teas with interesting health benefits with high customer attraction. *C. halicacabum shows the best potential among selected herbs.* However, it is suggested to carry out further studies to confirm these results and find out any adverse effects of overconsumption.

Keywords: α-Amylase inhibition; anti-oxidant; free sugar; phytochemicals; siddha medicine.

1. INTRODUCTION

Various types of medicinal plants are used in herbalism or herbal medicine. Herbs refer to different morphological plant parts including flowers, leaves, buds, stems, branches, rhizomes, seeds, fruits or roots [1]. The prehistoric ancient time people used fresh and dried herbs for the preparation of refreshing drinks and medicinal herbal infusions [2]. Although they depend on different theoretical, cultural, and religious principles in all models of traditional medicine such as Ayurveda, Siddha, Chinese, Unani, Tibetan, Amazonian, and African integrate phototherapy into their doctrine [3].

Naturally available plant-based products are used for chronic health problems such as high blood pressure and cholesterol [4]. It is known, that herbal tea reduces the adverse risk of non-communicable diseases, for example type-2 diabetes, cancer, brain dysfunction and auto immune diseases [5]. Furthermore, in contrast to chemical medicines, herbal treatments show minimal side effects to human health [6]. Therefore, herbal drugs constitute a major part in traditional medicine [7].

Herbs have a broad range of bioactive compounds (Predominant bio active components of herb extract and beverages) such as phenolic acids, polyphenols flavonoids, tannins, terpenoids and vitamins [5]. The herbal tea is prepared by pouring hot or boiling water on to the herbs. Due to the compound precipitation or micelles formation with gradual cooling of the solution, teas reduce the antioxidant capacity and polyphenol content of the extracts [8]. Herbs infusions prepared from most valuable parts of herbs, are among the major contributors of phenolic in our diet [9].

Sri Lanka has a long history of indigenous medicine which is dependent on medicinal Ayurvedic plants. Among the indigenous flora, over 30% are known to be having medicinal value [1]. Accordingly, this study has identified *Cardiospermum halicacabum* (Balloon vine), *Acalypha indica* (Indian copper leaf), *Ocimum tenuiflorum (*Tulsi or holy basil), *Solanum trilobatum (*Climbing brinjal) and *Plectranthus ambonicus (*Country borage) as underutilized medicinal herbs in Sri Lanka.

Although there are many natural herbal remedies with health benefits, they are not much popular among younger generations due to several reasons such as difficulties in finding them, preparations, and most of benefits are not scientifically proven. The major objective of this study was to identify the chemical properties such as total polyphenol content, anthocyanin content, free sugar content, antioxidant activity and the α-amylase inhibition in the selected medicinal herbal plants and to develop new herbal tea by catering the customer requirements and it is believed that the final product will be having minimal side effect, highly affordable and environment friendly.

2. METHODOLOGY

2.1 Sample Collection and Preparation

Leaf samples of herbs; *C. halicacabum* (Balloon vine), *A. indica* (Indian copper leaf), *O. tenuiflorum (*Tulsi or holy basil), *S. trilobatum (*Climbing brinjal) and *P. ambonicus (*Country borage) were collected randomly from Uva region in Sri Lanka. Collected wild grown samples were transported to the laboratory within 1 hour and washed thoroughly. Herbarium specimens were prepared by dipping in 70% methanol and drying. They were identified with

the help of reference specimens, herbarium of Royal Botanical Garden, Peradeniya, Sri Lanka. Samples were oven dried separately under 50 ℃ for 12 hours to remove moisture. Afterwards, each sample was powdered separately by mortar and pestle and sieved by1 mm mesh. Each powdered plant samples were weighed in to 5 samples (1 g-5 g) and boiled with 100 ml of boiling water (100 ℃) for 5 min. Infusions were filtered and residues were discarded.

2.2 Determination of DPPH Radical Scavenging Activity

Each solution (0.75 ml) was mixed with 0.3 ml of a 0.3 mmol dm^{-3} methanolic DPPH solution and kept in the dark at room temperature for 30 minutes. The absorbance was measured at 518 nm against a reaction blank. Antioxidant activity was calculated as ascorbic acid equivalents for each concentration and IC_{25}, IC_{50} and IC_{75}values were calculated for each extract. [10].

Percent inhibition = $\frac{Abs_{negative\ control} - Abs_{sample}}{A_{negative\ control}} \times 100$

2.3 α-Amylase Inhibition Assay

Dinitrosalicylic acid (DNSA) color reagent was prepared and α-Amylase (Type VI-B, porcine pancreas, 23 U /mg of protein) was prepared using phosphate buffer (pH 6.9). Absorbance was measured at 540 nm and percent inhibition was plotted against concentration to calculate IC_{25}, IC_{50} and IC_{75} [11].

Percent inhibition of α-amylase activity = $\frac{Abs_{negative\ control} - Abs_{sample}}{A_{negative\ control}} \times 100.$

2.4 Sensory Evaluation of Herbal Infusions

Sensory evaluation was conducted using 7.0-point Hedonic Scale based on ISO-3103 specifications with slight modifications. Selected sample weights were put into the cups and 180 ml boiling water (100℃) was added. Then, cups were covered using lid immediately. Subsequently, they were kept for 5 minutes and filtered using 1 mm filter. Finally, Sensory evaluation was done with the target group of 30 tasters considering colour, aroma, odour/smell, taste, appearance and overall acceptability of herbal infusions. Sample analysis was done using Microsoft Excel 2013 and Minitab 16 Statistical package.

2.5 Analysis of Other Chemical Properties

Total polyphenol content, anthocyanin content and free sugar contents of the samples selected from the sensory evaluation were tested. Infusion for each plant was prepared by boiling selected sample weights from sensory evaluation in 180 mL boiling water (100℃) for 5 minutes.

2.5.1 Determination of total polyphenol content

Total polyphenol content was determined by the Folin-Ciocalteu reagent method (ISO 14502-1: 2005) [12]. Absorbance of the samples were measured at 765 nm against distilled water as the blank. The total polyphenol content was determined as gallic acid equivalents (GAE).

2.5.2 Determination of anthocyanin content

The total anthocyanin content was determined by modified pH differential method [13] with slight modifications. The absorbance was measured at 510 and 700 nm against distilled water. Anthocyanin content was calculated ascyanidin-3-glucoside equivalents, using following equations.

Monomeric anthocyanin pigment (mg/L) $= (A \times MW \times DF \times 1000)/\varepsilon$

Where;[$= (A_{510} - A_{700})_{pH\ 1} - (A_{510} - A_{700})_{pH\ 4.5}$, ε = 26,900, DF=10].

2.5.3 Determination of free sugar content

Free sugar content was determined by Dinitrosalicylic Acid Method [14] with slight modifications. Absorbance was measured at 540 nm and free sugar content was calculated as glucose equivalents.

3. RESULTS AND DISCUSSION

3.1 Determination of DPPH Radical Scavenging Activity

Table 1 shows the IC_{50} value for DPPH radical scavenging activity of selected medicinal plants and it can be confirmed that *C. halicacabum, A. indica* and *S. trilobatum* have high radical scavenging capacity compared to the standard. Among them, the highest radical scavenging activity presents in *C. halicacabum* while

minimum has been noticed in *O. tenuiflorum*. If proper level of Antioxidant presents in particular herb, then it prevents human health from oxidative stress because this compound minimizes the oxidation [15].

The findings of this study supported the earliest findings reported by Kumaran and Joel Karunakaran in 2006 [16]. In their study, it has been found that the *C. halicacabum* in Methanolic extract has shown the significant result of Antioxidant Activity. However, they have tested in various in vitro methods in addition to DPPH, such as β-Carotene Linoleate Model System, Superoxide, Nitric oxide Radical Scavenging, and Reducing Power. Therefore, it can be concluded that there is the significant percentage of inhibition DPPH Radical Scavenging Activity present in *C. halicacabum* herb.

Further, results of this study revealed that the herb of *A. indica* also having Antioxidant Activity in considerable amount since the IC_{50} value was 10 mg ml^{-1}. Hence, above findings was aligning with previous findings with significant amount of Antioxidant Activity in different solvent like Hexane, Ethyl acetate, Acetone, Methanol and Water. Further, those studies investigated the herb by using different method namely Folin-Ciocalteu Assay (FC assay), DPPH Radical Scavenging Assay and a modified version of the Ferric Reducing Antioxidant Power Assay [17]. But, present study used leaves infusion only because in water the chemical Phenolic compound are highly present than the other solvent and results.

The findings assisted to the existing findings on Antioxidant Activity done by Fabiola and Sumathy in 2017 [18]. Present study has shown that the percentage of inhibition exists in between the range of 14.85%-59.9%. *S. trilobatum* herb at it also proved that there is the range of Antioxidant Activity presented above herb. Fig. 1 shows a graphical comparison of DPPH radical scavenging activities of selected herbs with standard.

3.2 Determination of α-Amylase Inhibition Activity

Table 2 shows the IC_{50} value for α-Amylase Inhibition Activity of selected medicinal plants and they are in the range of 4.2 mg ml^{-1} to 45.5 mg ml^{-1} while standard drug Acarbose IC_{50} value is 0.28 mg ml^{-1}. The maximum value of IC_{50} was recorded in *C. halicacabum* while minimum value of IC_{50} has been obtained in *A. indica.*

Comparing the current study's results with previous findings, one of the previous study shown that the Methanolic extract of *C. halicacabum* herb has the significant Antihyperglyceamic Activity at the dose of 200 mg/kg by decreasing the Plasma Glucose and increasing the Insulin and Hemoglobin level in blood [19]. Further, the in vitro α-Amylase Inhibition Activity of various extracts of *C. halicacabum* efficiently inhibits both α-Amylase and α-Glucosidase enzymes *invitro* in a dose dependent manner. In particular, Aqueous, Methanol and Heptane leaves and stem extracts of *C. halicacabum* represent maximum Antidiabetic Activity. [19]. Therefore, findings of this study verify and support those previous finding as *C. Halicacabum* has higher IC_{50} value of 45.5 mg ml^{-1}.

Moreover, the next herb which is *A. indica* shown the Antidiabetic properties of α-Amylase Enzyme Inhibition Assay in different solvent like Hexane, Ethyl Acetate, Acetone, Methanol and Water. Among those five solvents, there were a strong significant amount of inhibition in all solvent. The highest amount of *α*-Amylase Inhibition Activity was displayed by the Hexane Soxhlet and Ethyl Acetate Macerated Extracts while significant amount was identified in Water also. [17]. Therefore, it can be concluded that the findings of this study regarding the *α*-Amylase Inhibition Activity is aligned with the previous findings.

The next herbal plant taken for this study was S. *trilobatum.* One of the existing studies depicted that in the leaf extract of S. *trilobatum (*aqueous*)*, percentage of inhibition Antidiabetic activity was exist from 24 % to 57% in different concentration [18].

Further, the other two herbs used in this study were *O. tenuiflorum*, *P. amboinicus* and the IC_{50} value of both herbs were 7.3 mg ml^{-1} and 6.11 mg ml^{-1} respectively and which shows that both herbs are having *α*-Amylase Inhibition Activity. However, it was rare to find the previous evidence to prove these results. Fig. 2 shows a graphical comparison of α-Amylase Inhibition Activity of selected herbs with standard.

3.3 Sensory Evaluation of Herbal Tea Preparations

The purpose of sensory evaluation of the present study is to select the desirable weight of each

herb per 100 ml for consumption according to the organoleptic properties such as taste, aroma, colour and odour of herbal infusions. Accordingly, following hypothesis was derived to prove the differences among the selected five herbs.

H0: There are no any significant differences among the samples
H1: There are the significant differences among the samples

The tested sensory parameters of current study include colour, aroma, odour/smell, taste, appearance and overall acceptability. sensory ranking tests were performed by using 7.0 point Hedonic Scale to evaluate perceptible differences in the parameters of the samples. Accordingly, significant differences (α-.05) among the samples were found in terms of colour, aroma, odour/smell, taste, appearance and overall acceptability because p value is less than the significant level (p<.05).

3.5 g/100 ml infusion of *C. halicacabum* was identified as the best sample based on the colour (*P*=.000), aroma (*P*=.000), odour/smell (*P*=.000), taste (*P*=.000), appearance (*P*=.000) and overall acceptability (*P*=.000).

Table 1. IC50 values for DPPH radical scavenging activity of selected herbs

Herb/ Standard	IC_{50} value (mg ml^{-1})
C. halicacabum	7.71±0.58
A. indica	10.04±1.34
O. teniflorum	63.05±5.81
S. trilobatum	22.72±3.47
P. amboinicus	44.73±7.11
Ascorbic Acid (Standard)	42.59±2.45

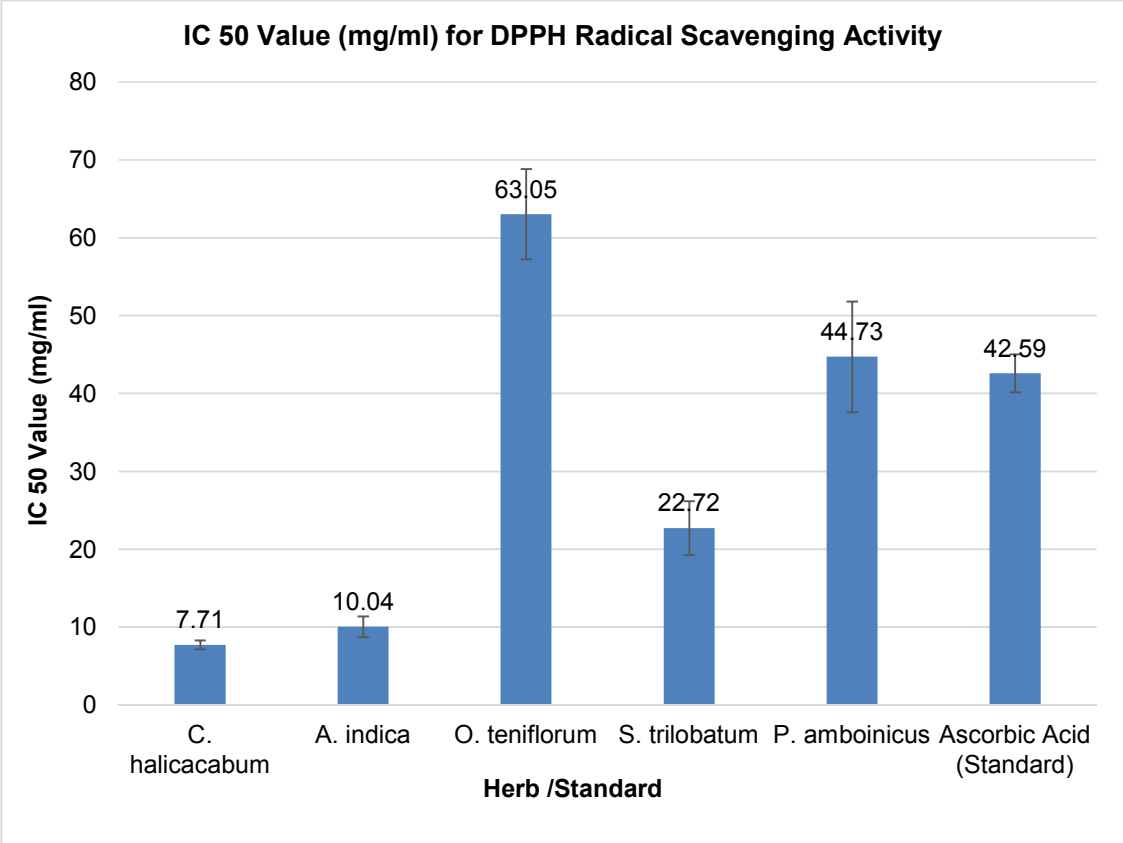

Fig. 1. IC50 values for DPPH radical scavenging activity of selected herbs

Table 2. IC_{50} value for α-amylase inhibition activity of selected herbs

Herb/ Standard	IC_{50} value (mg ml^{-1})
C. halicacabum	45.51±6.31
A. indica	4.24±0.61
O. teniflorum	7.35±0.34
S. trilobatum	5.23±0.44
P. amboinicus	6.11±1.20
Acarbose (Standard)	42.59±5.18

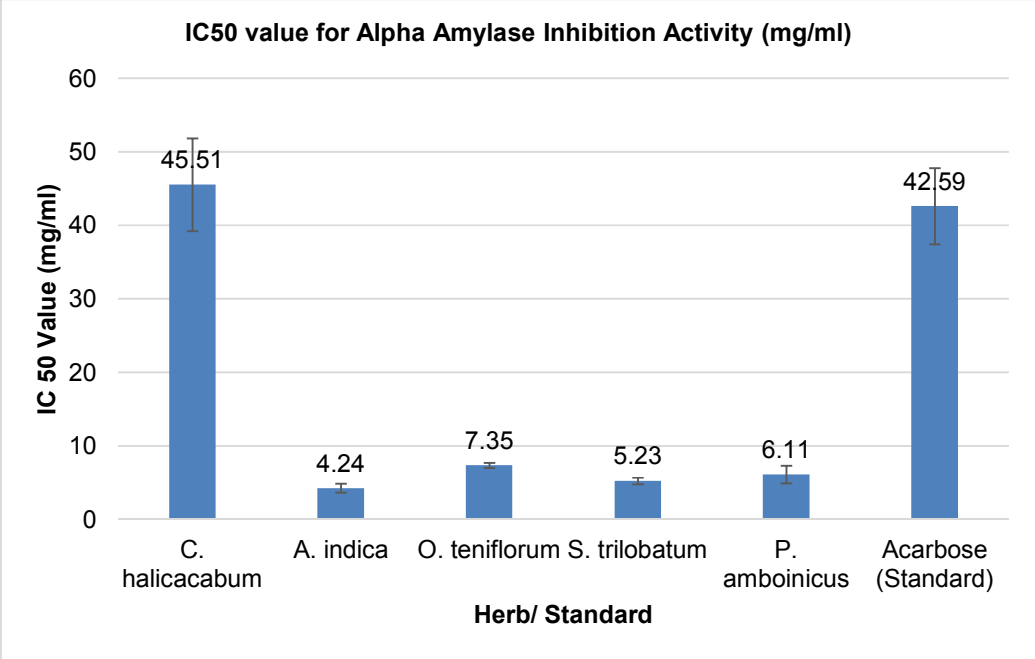

Fig. 2. IC_{50} value for α-amylase inhibition activity of selected herbs

Table 3. Summary of chemical analysis of selected herbs

Herb	weight used (g per 100 ml)	Chemical Analysis (ppm)		
		Total Polyphenol	Total Anthocyanin	Total Free Sugar
C. halicacabum	3.5	115.24±2.46	0.63±0.41	706.45±39.06
A. indica	3.0	193.14±1.02	0.33±0.24	788.86±34.76
O. teniflorum	2.0	236.65±0.38	8.41±0.79	57.23±0.04
S. trilobatum	1.0	120.49±2.07	3.06±0.27	24.67±0.58
P. amboinicus	3.0	127.46±1.91	0.51±0.12	97.85±0.95

3 g/100 ml infusion of *A. indica* was identified as the best sample based on the colour, aroma, odour/smell, taste, appearance, and overall acceptability as p value of all said parameters were *P*=.000.

2 g/100 ml infusion of *O. tenuiflorum* was identified as the best sample based on the colour ((*P*=..000), aroma ((*P*=.036), odour/smell ((*P*=.007), taste ((*P*=.000), appearance ((*P*=.001), and overall acceptability ((*P*=.000).

1 g/ 100 ml infusion of *S. trilobatum* was identified as the ideal sample based on the above parameters i.e. colour (*P*=.000), aroma (*P*=.000), odour/smell (*P*=.000), taste (*P*=..000), appearance (*P*=.000), overall acceptability (*P*=.000).

3 g/ 100 ml infusion of *P. amboinicus* was identified as the best sample based on the colour (*P*=.000), aroma (*P*=.001), odour/smell (*P*=.000), taste (*P*=.000), appearance (*P*=.000) and overall acceptability (*P*=.000).

3.4 Chemical Analysis of Herbal Tea Preparations

Herbal teas contain soluble Polyphenols which impart astringent effects thought to have medicinal properties. [20]. Furthermore, plant Phenolic compounds have the ability to act as Antioxidants, structural Polymers (Lignin), attractants (Flavonoids and Carotenoids), UV screens (Flavonoids), signal compounds (Salicylic Acid and Flavonoids) and defense response chemicals (Tannins and Phytoalexins) [21]. It is known that the Phenolic compounds are vital in defence responses, including Anti-aging, Anti-inflammatory, Anti-oxidant, Anti-diabetic and Anti-proliferative Activities. Thus, it is advisable and beneficial to eat herbal foods which have a high Antioxidant compound content and will cut down the incidence of certain Chronic diseases, such as Diabetes, Cancers and Cardiovascular diseases, through the management of oxidative stress [21].

Anthocyanidins and Anthocyanins are also used as natural dyes because they are colored pigments and having potential to use as pharmaceutical ingredients that give numerous beneficial health effects including Anti-diabetic, Anti-cancer, Anti-inflammatory, Anti-microbial, and Anti-obesity effects, as well as prevention of Cardiovascular diseases. [22].

Table 3 shows the total Polyphenol content, total anthocyanin content and total free sugar content in herbal preparations of each medicinal plant selected by consumers. Among the studied chemicals, polyphenols including anthocyanins have a direct relationship with antioxidant and enzyme inhibition properties, but the presence of high levels of free sugars might reduce their beneficial properties. Among them, *C. halicacabum* and *A. indica* preparations show high free sugar content compared to other herbs, which may not be advisable for consumers with a risk of type II diabetes. *O. teniflorum* preparation has best chemical properties for a herbal tea among these five herbs.

4. CONCLUSIONS

Selected herbs show positive responses towards selected biochemical properties and there is a very good potential to develop herbal teas with interesting health benefits with high customer attraction. However, it is suggested to carry out further studies to confirm these results and find out any adverse effects of overconsumption.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

Sensory evaluations of herbal tea preparations performed in studies involving human participants were in accordance with the ethical standards of the institution and followed according to ISO-3103 specifications. Informed consent was obtained from all individual participants included in sensory evaluation studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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